

Figure 2 a

## UNIT OPERATION

## PROCESS DESCRIPTION

## PROCESS CONTROLS

Frozen GHA Cell Paste



Cell Resuspension and Homogenization



Crude Lysate



Two-Phase System Make-Up and Partitioning

Bottom Phase  
? (Waste)



Top Phase



Top Phase Filtration



Follow procedure of U.S. Pat. No. 5,849,535 (recombinant expression of B-2036, centrifugate and freeze)

Cell Past Mass: 163-183 kg  
Buffer: 150 mM Tris and 5 mM EDTA, pH 7.2 (6.14 L/kg cell paste)  
Homogenizer Pressure: 900-1000 Bar  
Feed Temperature:  $\leq 15^{\circ}\text{C}$

Final Volume: ~1065 - 1136 L  
Monitor OD550 during cell resuspension

Conditioning: 125 grams ammonium sulfate per liter crude lysate  
125 grams PEG4600 per liter crude lysate  
Phase Contacting Temp:  $24-33^{\circ}\text{C}$   
Phase Contacting Time: 1-2 hours with mixing  
Separation Method: Continuous disk stack centrifuge  
Speed: 8340 RM  
Inlet Feed Rate: 7 LPM

Monitor conductivity of extract.

Dilution of Top Phase 1:1 with USP Purified Water  
Serial Filtration  
1) Charged delipidating depth (Seitz or equivalent)  
2) Charged cellulose depth (Seitz or equivalent)  
3) 0.2  $\mu\text{m}$  absolute cellulose acetate

Pre-Filter: LAL Bioburden, RPHPLC  
Post-Filter: LAL Bioburden, RPHPLC, IEXHPLC, HIHPLC  
Specific Recovery:  $\geq 2.5$  g B-2036 per kg cell paste

Figure 2 b

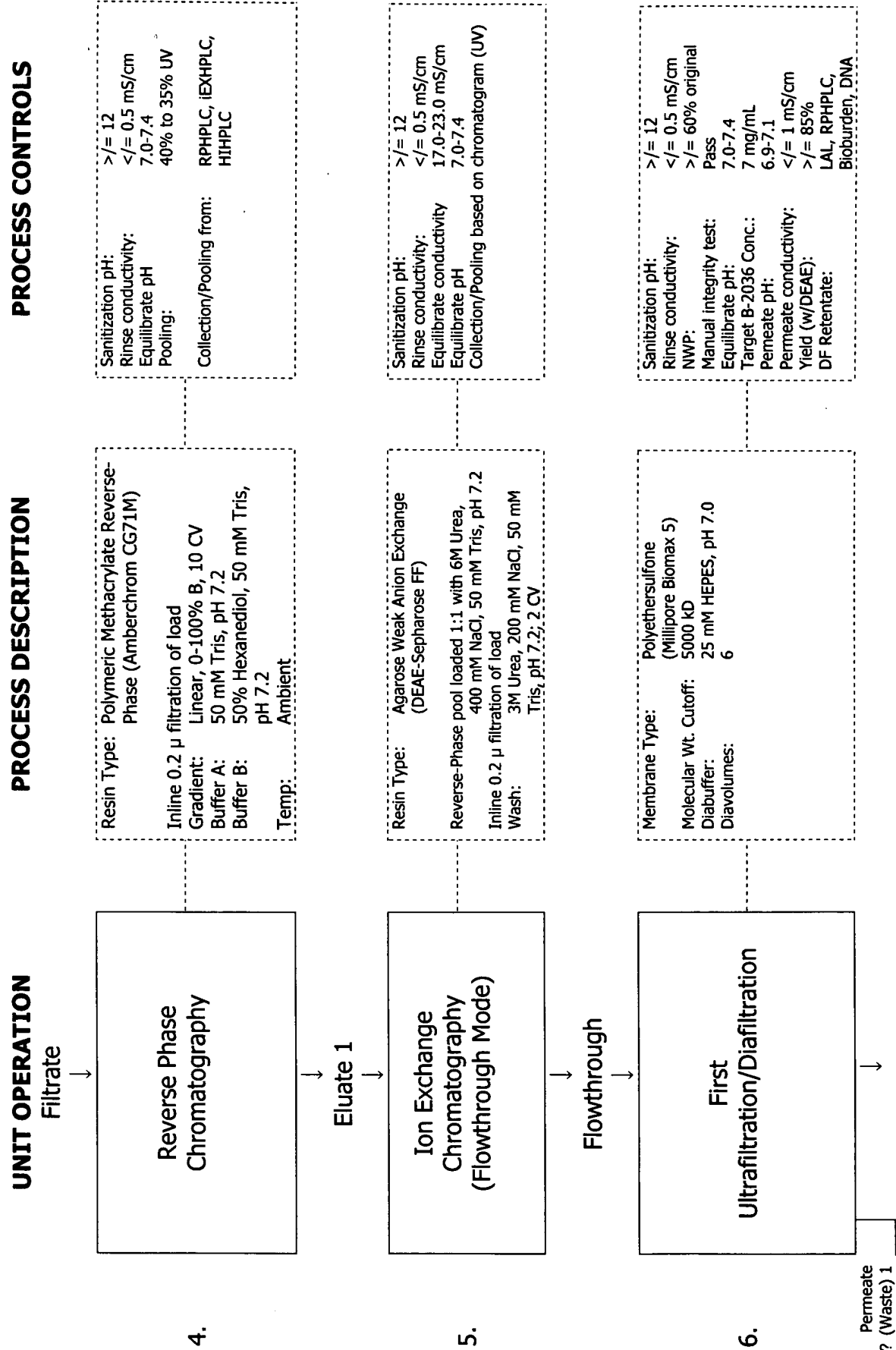


Figure 2 c

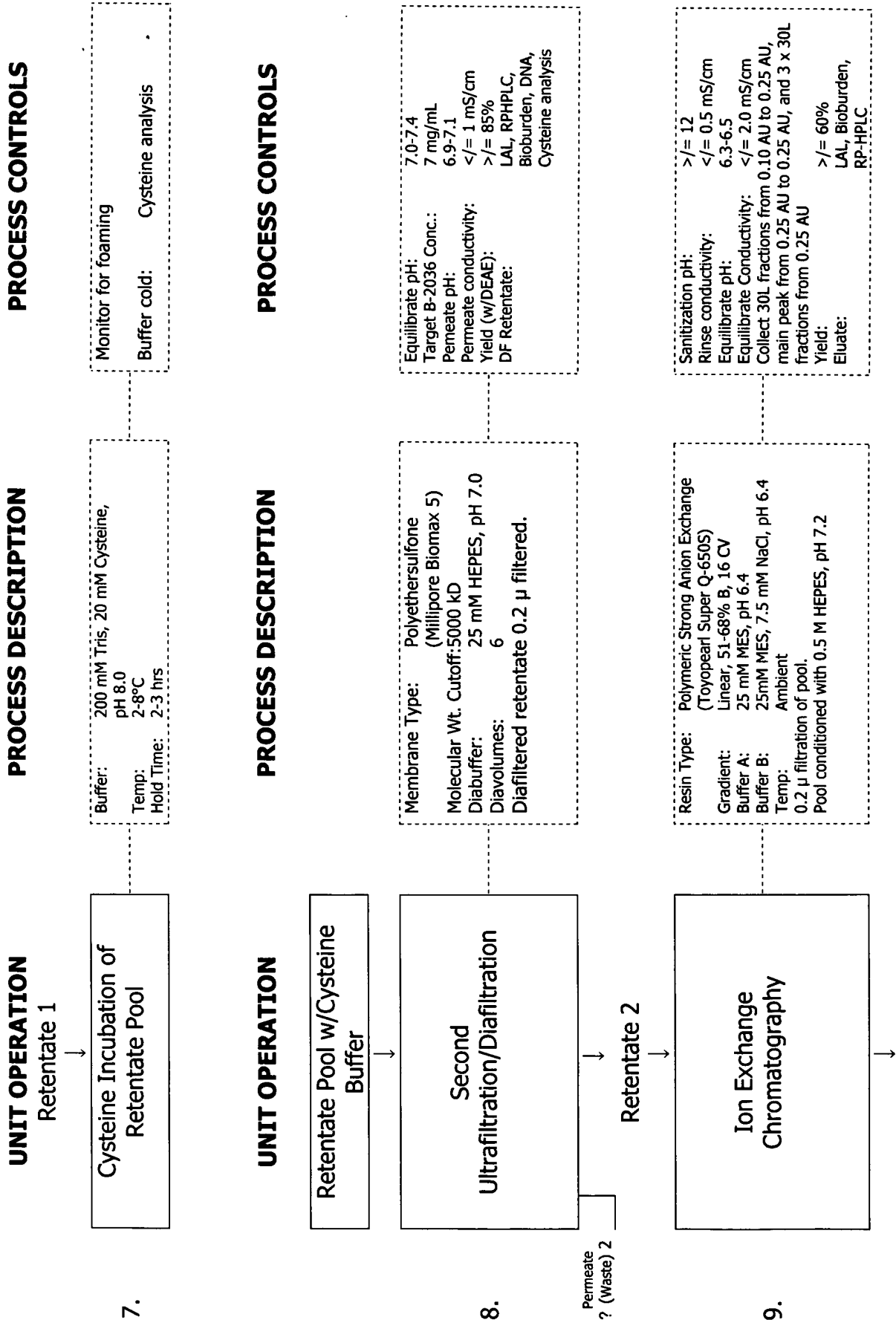


Figure 2 d

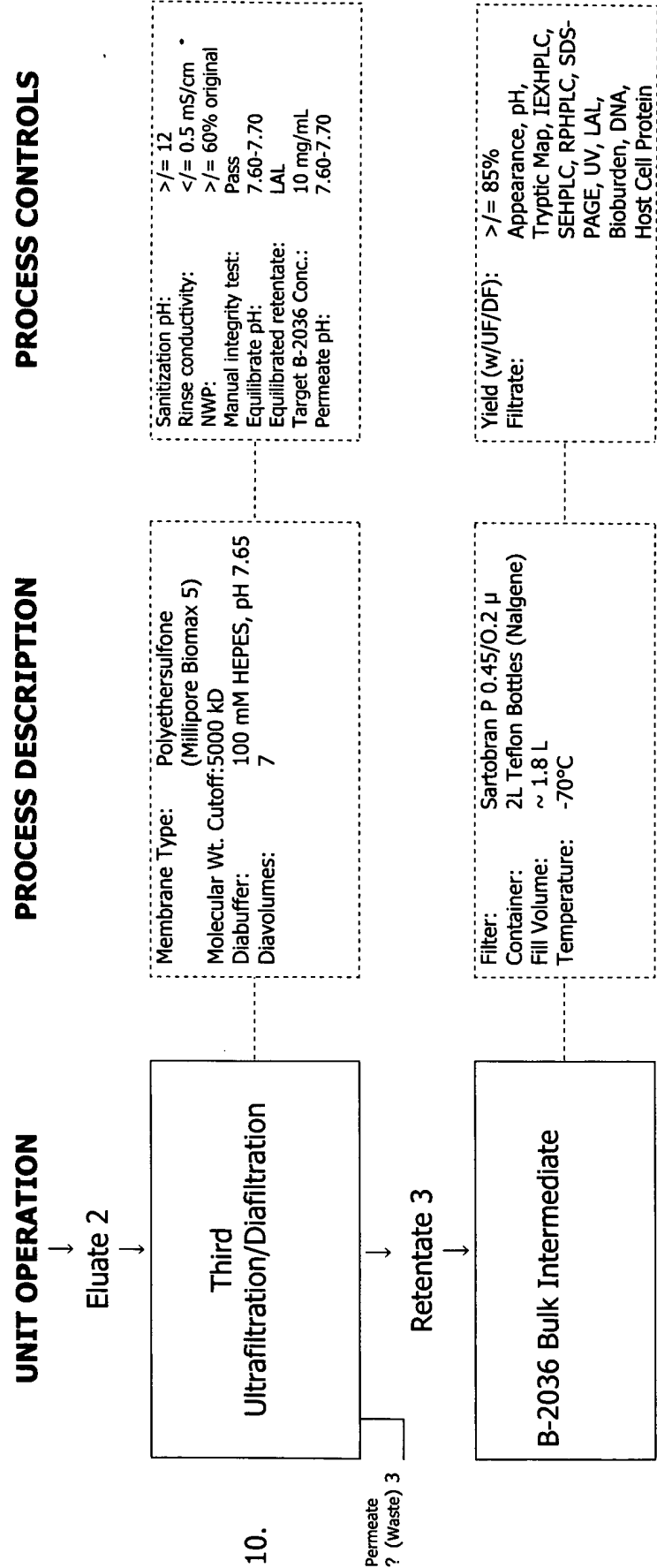


Figure 3 a

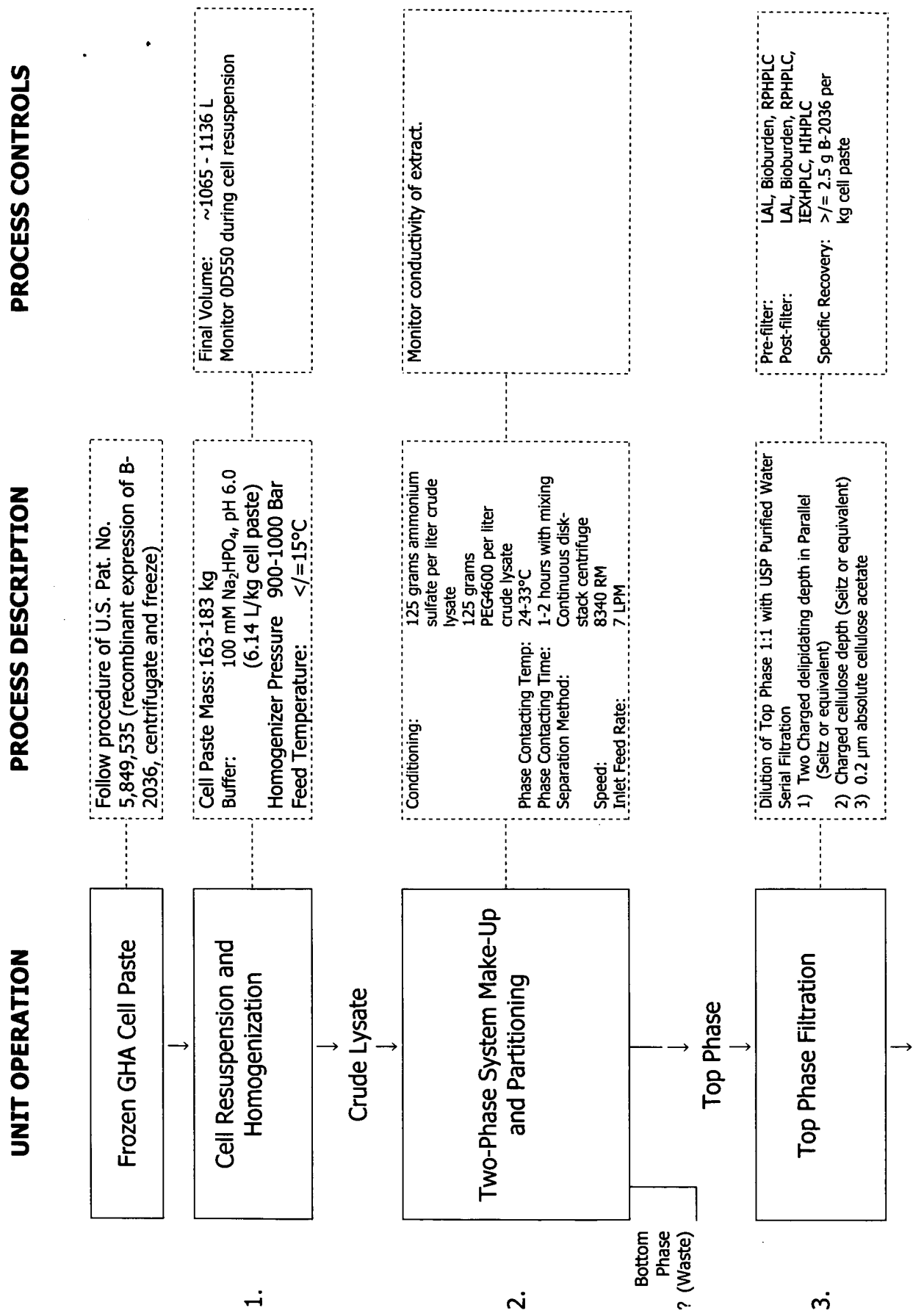


Figure 3 b

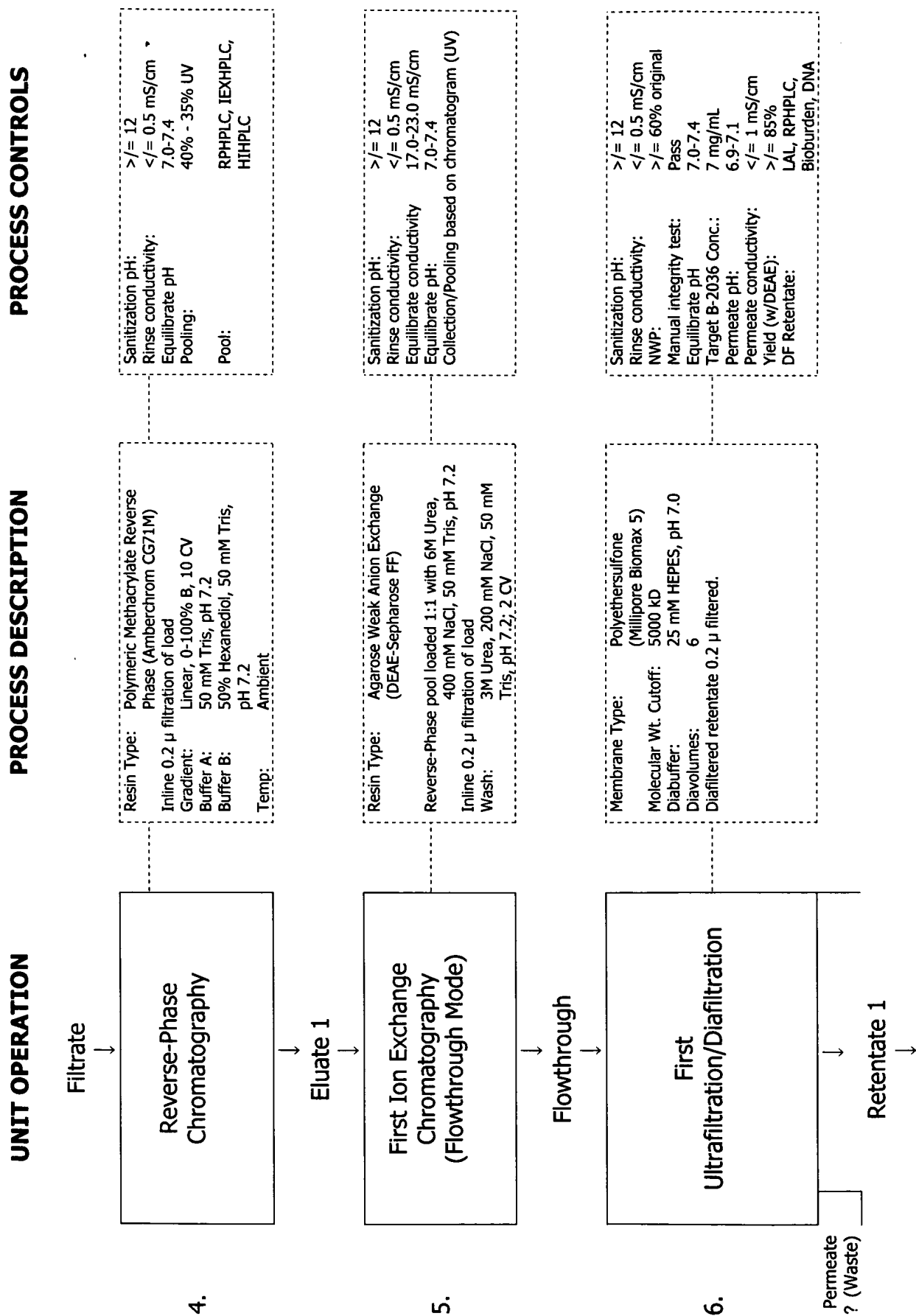


Figure 3 c

